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MODULATORY EFFECTS OF AN NMDAR PARTIAL AGONIST IN MK-801-INDUCED MEMORY IMPAIRMENT

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Abstract—*Rationale:* Acute administration of the N-methyl-D-aspartate (NMDA) non-competitive antagonist, MK-801, impairs novel object recognition (NOR), locomotor activity in open field (OF) and conditioned taste aversion (CTA) in rodents. NMDAR partial agonist D-cycloserine (DCS) reverses these effects in NOR and CTA via modulation of glutamatergic, cholinergic and dopaminergic systems. *Objectives and methods:* To test this hypothesis, we investigated the effects of DCS, a partial NMDAR agonist, on NOR memory, locomotor activity, and CTA memory in Wistar rats on NMDA-glutamate receptor antagonism by MK-801. The potential involvement of dopaminergic and cholinergic systems in improving cognitive functions was explored. MK-801-induced cognitive deficits were assessed using NOR, OF and CTA paradigms. MK-801-induced dopamine release increase in acetylcholinesterase (AChE), mono amine oxidase (MAO) activity and increase in *c-fos* expression were also investigated. *Results:* The effects caused by MK-801 (0.2 mg/kg) were inhibited by administration of the NMDA receptor agonist DCS (15 mg/kg). NOR and CTA paradigms inhibited by MK-801 were attenuated by DCS administration. Moreover, DCS also blocked the MK-801-induced abnormal increase in dopamine content, AChE activity and MAO activity. However, *c-fos* overexpression was controlled to some extent only. *Conclusions:* Based on the NMDAR hypo function hypothesis in some neuropsychiatric disorders, our finding suggests that improving NMDAR hypo function by agonist DCS may play a significant role.

Key words: NMDAR, Behavior, MK-801, D-cycloserine, Dopamine, *c-fos*.

INTRODUCTION

Learning and memory affect thinking, planning and decision making. They are attributed to changes in neuronal synapses. Investigating anatomical and physical paradigms of learning and memory is one of the significant achievements of modern neuroscience. Glutamate is the major excitatory neurotransmitter in the brain. One of its key neuronal functions involves learning and memory. Brain-derived neurotrophic factor (BDNF) enhances glutamate release and effects synaptic transmission and plasticity (Martin and Finsterwald, 2011). The N-methyl-D-aspartate receptors (NMDARs) are a major type of ionotropic glutamate receptors found in the brain. Ion flux through them is linked to neuroplasticity, learning and memory (Kehoe et al., 2013). NMDARs are crucial in various types of learning processes as they mediate long-term potentiation (LTP) which forms the foundation of memory (Luscher and Malenka, 2012). Ample evidence suggests that NMDAR antagonists are chemicals that deactivate the NMDAR by binding to glutamate or glycine and block the binding site of the neurotransmitter glutamate or glycine. They may also inhibit NMDARs by binding to allosteric sites or block the ion channel by binding to a site within it while agonists are chemicals that bind NMDAR and activate them. NMDAR agonists possess fast Mg^{2+} unbinding kinetics, increasing ion channel opening and depolarization. This nature is fundamental to the role of the NMDAR in learning and memory.

(+)-MK-801 hydrogen maleate is a ligand at phencyclidine i.e. PCP recognition sites associated with NMDAR-coupled cation channels where it acts as a potent non-competitive antagonist of central glutamate receptors. It elicits psychotomimetic and psychostimulatory effects, invoke locomotor hyperactivity, stereotype behavior, induce behavioral/cognitive deficits and alter working memory (Fowler et al., 2011; Olszewski et al., 2012; Karasawa et al., 2008; Csernansky et al., 2005; Zuo et al., 2006). After going through its complex mechanism of action, Farber et al. (2002) hypothesized that neurotoxic and psychotomimetic effects of MK-801 are mediated by glutamatergic and cholinergic receptor systems. Blockade of NMDA receptors in subcortical regions disinhibits cholinergic and glutamatergic projections to the cerebral cortex. Excitotoxic stimulation of glutamatergic receptors on cerebrocortical neurons is thought to be the possible mechanism for this. In addition, dopaminergic receptors have also been studied for causing psychosis and neurodegeneration

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Abbreviations: ACh, acetyl choline; AChE, acetylcholinesterase; ANOVA, analysis of variance; ATC, acetyl thio choline iodide; BAHC, benzyl amine hydrochloride; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; CTA, conditioned taste aversion; DAB, diaminobenzidine; DCS, D-cycloserine; DTNB, 5,50-dithiobis (2-nitrobenzoic acid); LTP, long-term potentiation; MAO, mono amine oxidase; NMDA, N-methyl-D-aspartate; NMDAR, N-methyl-D-aspartate receptor; NOR, novel object recognition; OF, open field; PCA, perchloric acid; PFC, prefrontal cortex.

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(Aosaki et al., 2010). Along with glutamate binding agonist sites, NMDARs also possess glycine binding co-agonist site to which glycine and D-serine can bind. Occupation of the glycine binding site is mandatory for channel stimulation by glutamate (Han et al., 2013). Glycine binding site has been recently shown to have a more natural affinity for amino acid D-serine (Wolosker, 2007).

D-Serine, an NMDAR competitive agonist has been shown to counteract memory loss following cortical damage in rats (Andersen et al., 2003) but NMDAR competitive agonists like D-serine causes excitotoxicity and cellular apoptosis (Canu et al., 2014). On the other hand, partial NMDAR agonists do not produce excitotoxicity (Mickleya et al., 2012). D-Cycloserine (DCS) is one such partial agonist that enhances excitatory NMDAR neurotransmission by binding to glycine NMDAR sites without causing excitotoxicity. It has been used to strengthen the experimental extinction of learned fear in rodents as well as humans (Ressler et al., 2004; Ledgerwood et al., 2005). Whenever endogenous levels of glycine or D-serine are less than optimal they limit NMDAR activation, exogenous DCS can then raise NMDAR activation to an optimal level of learning. DCS has been shown to be an effective memory enhancer in both pre-clinical and clinical studies (Mickleya et al., 2012). In the present work, we have shown how the combination of NMDAR antagonist and agonist influences cholinergic, glutamatergic and dopaminergic functions via neurobehavioral paradigms and how neurotransmission through these systems is crucial in pathophysiology of psychosis and other neurodegenerative disorders.

MATERIALS AND METHODS

Animals and housing

Female Wistar rats were used in this study. We used them, as they are known to be more sensitive than male rats toward the neurotoxic effects of NMDAR antagonists. All animals were maintained in groups of 4 at 12:12 light–dark cycle in a temperature and humidity controlled room, with food and water available ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (173/GO/Re/S/2000 CPCSEA). The animals were maintained under standard conditions in an animal house (Central Animal House Facility, Hamdard University, New Delhi) approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Drugs

(+)-MK-801 hydrogen maleate, acetyl thio choline iodide (ATC), benzyl amine hydrochloride (BAHC), bovine serum albumin (BSA), 5,50-dithiobis (2-nitrobenzoic acid) (DTNB), were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). DCS was purchased from SRL chemicals while Perchloric acid (PCA) was purchased from Merck Limited (Mumbai, India). The NR antagonist, MK-801 and the partial agonist of the glycine site of NR, DCS were diluted in physiological saline. The MK-801 was administered intraperitoneally at a dose of 0.2 mg/kg/ml (Karasawa et al., 2008) while DCS was administered intraperitoneally at a dose of 15 mg/kg/2 ml (Mickleya et al., 2012). At these doses, both MK-801 and DCS have been shown to influence behavior and learning processes.

Novel object recognition (NOR)

NOR was performed according to the method used by Karasawa et al. 2008 with certain modifications. Rats were habituated in the test chamber for 20 min. Next day, during training session, rats from all groups were placed back in the same chamber which contained two similar objects. Rats were allowed to freely explore the objects for 5 min and then were returned to their home cages. DCS was administered 10 min before MK-801 and test was performed 20 min later. During test, one previously explored object was replaced with a novel object (with a different color & shape). Rats were placed back in the testing chamber with the novel object and one previously explored object/familiar object for 5 min. Rats were evaluated for their memory to remember the familiar object. A digital camera was mounted on the ceiling of the chamber. All equipment was cleaned with 70% ethanol between each session to escape odor cues. Object exploration was measured by Any-maze software and was defined as the time rats were in direct contact with the object. Exploration time was measured as the amount of time spent exploring any one of the two objects (during the training session) or the replaced novel object (during the test session) over the total time spent exploring both objects.

Open field (OF)

A sheet of paper with a grid pattern was placed on the floor of the apparatus. The grid subdivided the OF into nine squares. The grid-printed paper floor was changed after each subject was tested, eliminating any odor cues within the apparatus. Each rat was placed individually in the OF for 5 min for testing locomotor activity. The number of line crossings was recorded by using Any-maze software.

Conditioned taste aversion (CTA)

CTA was performed according to Ferreira et al. (2002) with certain modifications. Animals were deprived of water for 24 h and then acclimated to drinking from the graduated tubes for 3 days to obtain their daily water within 15 min in their home cages and water consumption was measured. Water was provided at the same time every day. On the conditioning day, animals were presented with access to 0.1% saccharin (CS), 20 min after MK-801 injection and 30 min after DCS administration. Immediately, after consumption of saccharin, 0.4 M LiCl (US) was injected intraperitoneally (7.5 ml/kg). The animals were observed for behaviors that represent internal malaise (e.g. "lying on belly"). Four hours after LiCl injection, rats were exposed to saccharin solution for 15 min to assess the memory of CTA. CTA strength was measured